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Effect of soil properties on Pb bioavailability and toxicity to the soil invertebrate *Enchytraeus crypticus*



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HIGHLIGHTS

- Pb toxicity to *Enchytraeus crypticus* was determined in six different natural soils.
- Soil properties highly affected Pb availability, uptake and toxicity to *E. crypticus*.
- CaCl_2 extractable Pb best explained Pb bioaccumulation and toxicity in all soils.
- $\text{pH}_{\text{CaCl}_2}$ was main factor predicting Pb toxicity based on total soil concentrations.

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ABSTRACT

The present study investigated the bioavailability and toxicity of lead to the potworm *Enchytraeus crypticus* in six soils with different properties. Pb partitioning between the soil solution and solid phase was affected by soil organic matter (OM) content, cation exchange capacity (CEC) and water holding capacity (WHC). After 21 d exposure, Pb bioaccumulation in the enchytraeids was positively correlated with total soil Pb concentration. Bioaccumulation was best predicted by Pb availability (CaCl_2 -extractable and porewater Pb concentrations), and by the Ca concentration in pore water and the CEC of the soils. Toxicity varied greatly among soils, with LC_{50} s and $\text{EC}_{50_{\text{reproduction}}}$ s based on total Pb concentrations ranging from 246 to >3092 and from 81 to 1008 mg Pb/kg dry soil, respectively. The variation in LC_{50} s among soils was explained by differences in CaCl_2 -extractable Pb concentrations in soil and internal Pb concentrations in the animals. The differences in $\text{EC}_{50_{\text{reproduction}}}$ s could be explained from the CaCl_2 -extractable Pb concentrations in the soils. Although it was also correlated with CEC and porewater Ca concentration, $\text{pH}_{\text{CaCl}_2}$ was the dominating factor for predicting Pb toxicity based on total soil concentrations. This study demonstrates that soil properties, such as pH, CEC and Ca concentration in pore water, significantly affected the bioavailability and toxicity of Pb and therefore should be taken into account when assessing the ecological risk of metals in contaminated soils.

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1. Introduction

With the rapid development of society and economy, anthropogenic activities such as agriculture, industrialization, urbanization and mining are contributing to the emission of lead (Pb) to the soil (Facchinelli et al., 2001). This leads to accumulating exposure of the receiving environment, posing the risk of causing detrimental effects to ecosystems. This may also threaten the health of animals and human beings through the food chain, which may bring high costs for soil remediation and re-development. As a consequence,

there is increasing concern on the environmental risks associated with soil Pb pollution.

Currently the legislation and Pb assessment mainly focus on total Pb concentrations in the soil. However, only a fraction of the total metal concentration in the soil is available for uptake and causing toxic effects in soil organisms, which is defined as metal bioavailability (Peijnenburg et al., 2007). Bioavailability of Pb in soil depends on soil properties (Luo et al., 2014ab) and an evaluation of Pb toxicity based on total metal concentrations, therefore, might over or underestimate the actual availability and risks of Pb in the soil. Bioavailability results in bioaccumulation but could not be translated into adverse effects as it contains the processes involved in the flux of metals to the biological target sites. To assess the chemical availability of metals in soil, various methods and

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sampling strategies have been developed. Extractants (e.g. water, weak salt solutions) have been suggested for the prediction of metal availability in soil, providing a first indication of the potential threat of a metal (Conder and Lanno, 2000; Peijnenburg et al., 2007). It is commonly assumed that pore water is the dominant uptake route for soil invertebrates (Van Gestel, 2012). Metal concentration in the pore water, therefore, might be a good indicator for metal bioavailability in soils. In addition, a combination of chemical and biological tests may be more efficient and accurate to provide information on metal bioavailability in soils (Antunes et al., 2008; Van Gestel, 2012; Smith et al., 2012).

Standardized laboratory toxicity test (e.g., OECD, ISO) are the basis for the environmental risk assessment of chemicals in terrestrial ecosystems. The physicochemical properties of the soil (e.g. pH, organic matter content and cation exchange capacity (CEC)) are important factors modifying metal bioavailability to soil organisms and subsequently affecting metal toxicity. The variation in soil properties might lead to significant differences in metal bioavailability and toxicity in soil. Bradham et al. (2006) found large differences in Pb toxicity to earthworm survival in different soils and concluded that soil properties had a great influence on its bioavailability to ecological receptors. Soil pH was the most dominant soil parameter attributing most to the difference in toxicity. In 20 contaminated soils, Peijnenburg et al. (1999a) demonstrated that pH was the dominant soil property determining Pb uptake in earthworms. Luo et al. (2014a) also found that soil properties significantly affected Pb bioavailability and toxicity, with collembolans being more sensitive to soil pH rather than Pb content when exposed to six polluted soils from a shooting range. Furthermore, pH, organic matter content and CEC were reported to be the soil properties mostly influencing Pb survival to *Enchytraeus albidus* (Lock and Janssen, 2001). However, it is very hard to determine the effect of individual soil properties on metal bioavailability and toxicity because of their interactions. In addition, from these studies, it remains unclear how soil properties do affect sublethal endpoints and what is the best measure of metal bioavailability in soil. Assessing metal toxicity in soils with different physicochemical properties to terrestrial organisms may help improving our understanding of the influence of soil characteristics on metal bioavailability and reproduction toxicity in soil.

This study was designed to unravel the impact of soil properties on Pb bioavailability and toxicity to *Enchytraeus crypticus*, which was recommended as a test species in ecotoxicological tests. We aimed at: (1) assessing the sorption of Pb to soils with different properties, (2) determining Pb bioaccumulation and its lethal and sub-lethal (reproduction) toxicity in soils with different properties, (3) linking Pb toxicity to chemical and biological measures of Pb bioavailability, and (4) relating Pb bioaccumulation and toxicity to soil properties (e.g., organic matter content, cation exchangeable capacity and pH).

2. Materials and methods

2.1. Test organism

Enchytraeus crypticus (Enchytraeidae; Oligochaeta; Annelida) has been cultured for several years in the laboratory of the Department of Ecological Science, Vrije Universiteit, Amsterdam. *E. crypticus* were kept on agar prepared with an aqueous soil extract, in a climate room at 16 °C, with 75% relative humidity, and in complete darkness. The animals were fed twice a week with a mixture of oat meal, dried yeast, yolk powder, and fish oil (Castro-Ferreira et al., 2012). Adult *E. crypticus* of approximately 1 cm were used in the tests.

2.2. Test soils

Six natural soils with different properties were selected in this study. Standard soils (LUFA 2.1, LUFA 2.2, LUFA 2.3, LUFA 2.4 and LUFA 5 M) were obtained from the Landwirtschaftliche Untersuchungs-und Forschungsanstalt (LUFA) at Speyer, Germany. A grassland soil was collected from a soccer field at De Kwakel, Netherlands. The soils were homogenized, sieved through a 5 mm mesh and air dried at 40 °C before use in the experiments.

Soil organic matter (OM) content was determined as loss on ignition at 500 °C. Soil pH_{CaCl2} was measured using a pH meter (WTW, Inolab pH7110) in 5:1 solution:soil slurries, which was incubated overnight after 2 h shaking (200 rpm). To assess the 0.01 M CaCl₂-extractable Pb content of the soils, the supernatants were filtered over a 0.45 µm cellulose nitrate membrane filter (Whatman). The cation exchange capacity (CEC) of the soils was determined by the Silver Thiourea Method, using diluted unbuffered silver-thiourea (AgTU) solution (0.01 M Ag⁺) (Dohrmann, 2006). To determine the maximum water holding capacity (WHC), soil samples were saturated with water in a cylinder placed in quartz sands until the excess water was drawn away by gravity. WHC was calculated based on the weight of the water held in the sample vs. the sample dry weight. To obtain pore water, soils were saturated with deionized water to 100% WHC and equilibrated for a week at room temperature. Then the samples were centrifuged at 2000 rpm and 16 °C for 45 min (Centrifuge Falcon 6/300) over a 0.45 µm membrane filter (Whatman) that was placed in between two filter papers (Whatman). The dissolved organic carbon (DOC) content in the pore water was measured by total organic carbon analyzer (TOC-5000, Shimadzu, Japan).

To obtain nominal concentrations of 0, 100, 200, 400, 600, 800, 1200, 1600, 2400 and 3200 mg Pb/kg dry soil, soils were spiked with aqueous solutions of Pb(NO₃)₂ (purity >99.99%; Sigma-Aldrich; USA). For each treatment, the soil was moistened to reach 50% WHC. The spiked soils were equilibrated for 14 d in a climate room at 20 °C before use in the tests.

2.3. Toxicity tests

OECD guideline 220 (OECD, 2016) was followed to determine the effect of Pb(NO₃)₂ on the survival and reproduction of *E. crypticus*, with modifications following Castro-Ferreira et al. (2012). Five replicates were used for each treatment and control. For each replicate, ten adult potworms were introduced into a 100 mL glass jar with 30 g moist soil after which 2 mg oatmeal was added for food. Test jars were covered with perforated aluminum foil and kept in a climate room at 20 °C, 75% relative humidity and a light/dark photoperiod cycle of 16/8 h. Once a week, food and moisture content of the soil were checked, and the water loss was replenished by adding deionized water when necessary. After 21 d, the soil from each test jar was transferred to a 250 mL plastic salad box. Surviving adults were collected and placed in a plastic container filled with 5 mL ISO solution containing 294 mg/L CaCl₂·2H₂O, 123.3 mg/L MgSO₄·7H₂O, 5.8 mg/L KCl and 64.8 mg/L NaHCO₃ (Sigma Aldrich, *99%) (ISO, 2012) for gut depuration for 24 h. Afterwards, three worms from each replicate were stored at −20 °C for further analysis. The jars were rinsed with 10 mL ethanol (VWR chemicals, 96%) to fixate the remaining worms, which were extracted as described by Zhang and Van Gestel (2017a). Juvenile numbers were counted on pictures taken using Photoshop CS5.0.

2.4. Chemical analysis

After 2 weeks equilibration, soil samples were dried at 40 °C. For

the determination of total Pb concentrations, soils were digested in a mixture of HNO₃ (65%, Sigma-Aldrich, USA) and HCl (37%, Sigma-Aldrich, USA) (4:1 v/v). About 130 mg dry soil was mixed with 2 mL of the acid mixture in a Teflon container which was tightly closed and heated for 7 h at 140 °C in an oven (Binder FD). Total soil concentrations were measured by flame atomic absorption spectrometry (AAS; AAnalyst 100, Perkin Elmer, Germany). The certified reference material ISE sample 989 (International Soil-Analytical Exchange) was included for quality control in this study and the recoveries of Pb in the reference material were 92.8–96.7%. The detection limit for Pb analysis by flame AAS was 0.027 mg/L.

The frozen potworms were freeze-dried for at least 24 h, individual dry weight determined using an analytical balance (Mettler Toledo GmbH, 1998) and digested with 300 µL mixture of HNO₃ (65%; Mallbaker Ultrex Ultra-Pure) and HClO₄ (70%; Mallbaker Ultrex Ultra-Pure) (7:1 v/v) in a block heater (TCS Metallblock Thermostat) with a heating ramp ranging from 85 to 180 °C for 2 h. Afterwards, the Pb concentrations in the potworms were measured by graphite furnace AAS (PinAAcle 900Z, Perkin Elmer, Germany). The quality of the analysis was checked by using the certified reference material DOLT 4 (Dogfish liver, LGC Standards). The Pb recoveries were 89.2–97.9%. The detection limit for Pb in this analysis was 0.21 µg/L.

The Pb concentrations in the pore water and in the 0.01 M CaCl₂ extracts were determined by flame or graphite furnace AAS depending on the concentration level. Ca concentrations in the pore water were measured by flame AAS (AAnalyst 100, Perkin Elmer, Germany).

2.5. Data analysis

The Pb²⁺ free ion activity in the pore water were calculated using Visual MINTEQ 3.0 (Gustafsson, 2011), based on pH_{pw}, and DOC, Ca and Pb concentrations measured in the pore water.

The sorption of lead to the test soils was described by the Freundlich isotherm (Eq. S1). The relationship between Pb uptake by the test organisms and soil Pb concentrations could be described by a Langmuir isotherm (Eq. S2). To take into account the effect of competing cations on Pb uptake, a modified Langmuir model was used (Eq. S3).

The effect concentration L/EC_x (mg/kg or mg/L) associated with x% reduction in survival or reproduction compared with the control was estimated by a logistic dose-response model. To determine differences in L/EC_x values between soils, a generalized likelihood ratio test was used. To assess the potential effect of soil characteristics, sorption (K_F), uptake in *E. crypticus* and toxicity of Pb (LC50, LC10, EC50 and EC10 based on total Pb concentrations in the soil) were related to soil properties. First, Pearson correlation analysis was performed to identify the most significant soil properties for Pb sorption and toxicity. Second, simple linear regression was used to relate Pb sorption and toxicity to these parameters. Third, multiple stepwise regression was carried out to quantitatively analyze the combination of soil properties affecting Pb sorption, accumulation and toxicity. Soil pH_{CaCl2}, pH_{pw}, porewater Ca and porewater DOC data for the individual spiked treatments were used in the multiple regressions for Pb bioaccumulation. Since correlation analysis of toxicity data based on extractable concentrations did not yield significant outcomes, data are not shown here.

In this study, all calculations used measured concentrations and all parameters mentioned were estimated by regression analysis in SPSS 24.0.

3. Results

3.1. Soil properties

The properties of six test soils are shown in Table S1. The measured OM content ranged from 1.27% in LUFA 2.1–12.8% in Grassland soil. The DOC concentration in pore water increased linearly with increasing OM content from 46 mg/L in LUFA 2.1–189 mg/L in Grassland soil ($R^2 = 0.975$, $p < 0.01$) (Fig. S1). Among the soils.

Studied, LUFA 2.1 had the lowest CEC, while LUFA 2.4 and Grassland soil had the highest CEC (20 cmol_c/kg). A positive linear correlation was found between CEC and OM content ($R^2 = 0.663$, $p < 0.05$) (Fig. S1). LUFA 2.1 had the lowest pH_{CaCl2} (4.86), followed by LUFA 2.3 and LUFA 2.2; LUFA 5 M, LUFA 2.4 and Grassland soil the highest pH_{CaCl2} (6.85–6.99).

3.2. Soil pH, total Pb and extractable Pb concentrations

For all test soils, soil pH_{CaCl2} and pH_{pw} in the pore water decreased with increasing total Pb concentration in the soil (Tables S2 and S3). In LUFA 2.1 and LUFA 2.3, pH_{CaCl2} decreased by approx. one unit at the highest exposure concentration and pH_{pw} decreased by about 2 units, while in LUFA 2.2, LUFA 2.4, LUFA 5 M and Grassland soil, pH values slightly declined. pH reduction in pore water was much more pronounced than in the CaCl₂ extracts.

The measured total Pb concentrations in the test soils are shown in Table S4. Total Pb concentrations in the un-amended soils ranged between 10 and 32 mg Pb/kg dry soil. Pb recovery in the spiked soils ranged between 93% and 117%.

CaCl₂-extractable Pb concentrations increased with increasing total Pb concentrations (Table S5). In LUFA 2.1 and LUFA 2.3 the CaCl₂-extractable Pb concentrations exceeded 30% of the total Pb concentration at the highest contamination level. The lowest CaCl₂-extractable Pb concentrations were measured in LUFA 2.4 and Grassland soil, corresponding with less than 0.5% of the total spiked Pb concentration. The Freundlich isotherm described the relationship between total Pb and CaCl₂-extractable Pb concentrations in the soil well for all soils with $R^2 > 0.98$ (Table S6). The lowest Freundlich sorption constant (K_{FC}) value of 337 (L/kg)ⁿ ($n = 0.434$) was estimated for LUFA 2.1, while the highest K_{FC} (7386 (L/kg)ⁿ ($n = 0.561$)) was found for the Grassland soil. For all the spiked soils, the shape parameter n was lower than 1. Pearson correlation analysis identify OM content, CEC and WHC as the significant factors for Pb sorption, with OM content being the dominant parameter according to simple and stepwise regression analysis (Table S7).

Porewater Pb concentrations increased with increasing total Pb concentrations in the soil (Table S8). The relation between total soil Pb concentration and porewater Pb concentrations was fitted very well with Freundlich isotherms with $R^2 > 0.90$ (Table S6). The lowest K_{FP} (361 (L/kg)ⁿ ($n = 0.410$)) was calculated for LUFA 2.1, the highest K_{FP} (9768 (L/kg)ⁿ ($n = 1.22$)) for LUFA 2.4. For all soil types except LUFA 2.4, the shape parameter n was lower than 1. CEC and pH_{pw} were the most significant parameters for Pb sorption (Pearson correlation analysis). Together with WHC, CEC explained 99.2% of the variance in K_{FP} calculated in stepwise regression analysis (Table S7).

The Pb²⁺ free ion activities in the pore water calculated using Visual MINTEQ 3.1 (Table S9) increased with increasing total Pb concentration in the soil. Freundlich isotherms very well fitted the relation of total soil Pb concentrations with the Pb²⁺ free ion activities with $R^2 > 0.88$ (Table S6). LUFA 2.1 had the lowest sorption, while the highest sorption was found in LUFA 2.4. CEC was the most significant parameter explaining 81.5% of the variance in K_{FF} in

stepwise regression analysis (Table S7).

3.3. Pb bioaccumulation

The average internal Pb concentration in *E. crypticus* increased from 0.5 to 2.7 to levels up to 47.7–110 mg Pb/kg dry body wt after 21 d of exposure (Fig. 1). As no surviving adults could be found at the concentrations higher than 229, 1558 and 780 mg Pb/kg dry soil in LUFA 2.1, LUFA 2.2 and LUFA 2.3 after 21 d of exposure, respectively, no body Pb concentration could be determined at these exposure concentrations. When exposed to the same external Pb concentration in the soil, the lowest Pb uptake in enchytraeids was found in Grassland soil, the highest in LUFA 2.1. Pb concentrations in the surviving animals increased with increasing exposure concentrations, and showed linear patterns when data for individual soils were related to total soil concentrations (Fig. 1A). When fitting a Langmuir isotherm to all data together, R^2 was 0.647 and the estimated maximum uptake capacity was 79.4 mg Pb/kg dry body wt (Fig. S2). CaCl_2 -extractable and porewater Pb concentrations best predicted body Pb concentrations in *E. crypticus* (Table 1), and overall Langmuir models could well describe internal Pb concentrations from all test soils, with estimated maximum uptake capacity of 82.9 and 95.7 mg Pb/kg dry body wt, respectively

($R^2 > 0.814$) (Fig. 1B and C). Pb^{2+} free ion activity in pore water did not explain internal concentrations in the enchytraeids (Fig. S3). Correction for H^+ activity however, gave a much better estimation of body Pb concentrations using a Langmuir model (Fig. 1D). This estimation did not improve when considering Ca^{2+} activity in the pore water. Pb bioaccumulation was best predicted by the combination of total Pb concentration, soil pH, CEC, WHC and OM content, explaining 92.2% of the variance (Table 1).

3.4. Toxicity

The control performance of the enchytraeids was affected by soil type. The adult mortality in all control soils was less than 5%. The average juvenile numbers in the control were between 128 and 708, with coefficients of variation between 4.8% and 16.4%, so meeting the validity criteria set by (OECD, 2016).

The effect of $\text{Pb}(\text{NO}_3)_2$ on the survival of *E. crypticus* after 21 d of exposure in the six test soils is shown in Fig. 2. No significant adult mortality was observed in Grassland soil, thus no LC50 or LC10 could be estimated. Adult survival declined in a dose-dependent manner in the other soils, which was well described by logistic dose-response models with $R^2 > 0.94$. LC50s ranged from 246 mg Pb/kg dry soil in LUFA 2.1 to >3092 mg Pb/kg dry soil in

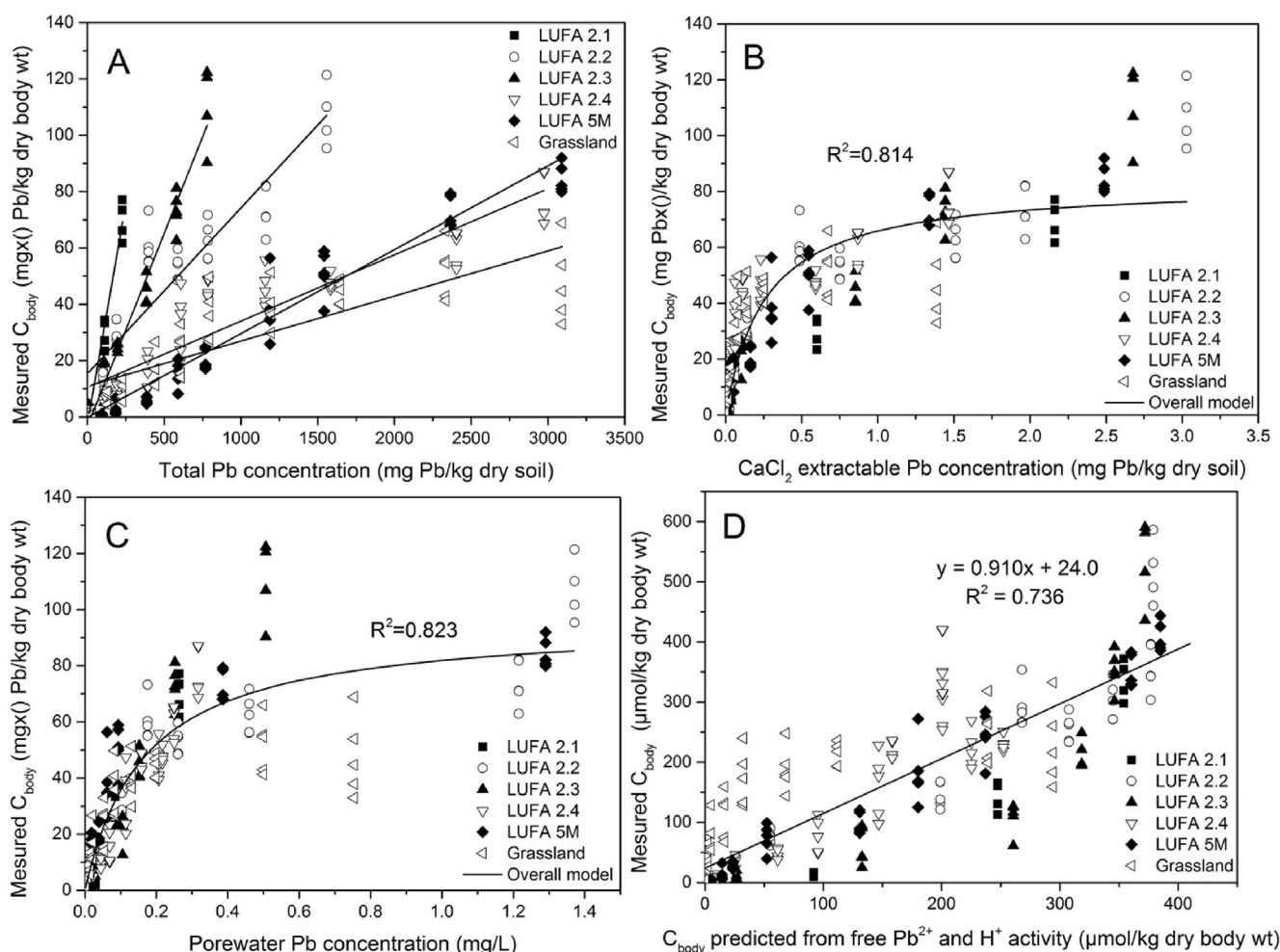


Fig. 1. Lead concentrations in surviving adult *Enchytraeus crypticus* (C_{body}) measured after 3 weeks exposure to $\text{Pb}(\text{NO}_3)_2$ in six natural soils with different soil properties, related to total (A) and 0.01 M CaCl_2 -extractable Pb concentrations in soil (B), Pb concentrations in pore water (C), and to internal concentrations predicted from Pb^{2+} free ion activity in pore water with correction for H^+ activity using a Langmuir model (D). Symbols represent measured concentrations, lines show a linear fit (A) or the fit of a Langmuir isotherm (Eq. S2) to the data (B, C). C_{body} is in mg/kg for figures A–C, but in $\mu\text{mol/kg}$ for figure D.

Table 1

Simple and multiple linear regressions for the relationship between Pb bioaccumulation in *Enchytraeus crypticus* (C_{body} ; $n = 233$) exposed for 21 days to $Pb(NO_3)_2$ in six different natural soils and soil physicochemical properties. R^2 (adj) presents the percentage of variation explained by only the independent variables that actually affected the dependent variable. See Fig. 1 for Pb accumulation, Tables S3–6 for Pb concentrations and Table S1 for soil properties.

Equation	R^2 (adj)	p
$\log C_{body} = -0.669 + 0.761 \log [Pb]_{total}$	0.698	<0.001
$\log C_{body} = 1.79 + 0.510 \log [Pb]_{CaCl_2 \text{ extractable}}$	0.795	<0.001
$\log C_{body} = 2.16 + 0.767 \log [Pb]_{pw}$	0.819	<0.001
$\log C_{body} = 2.34 + 0.240 \log [Pb]_{free}$	0.460	<0.001
$\log C_{body} = 5.83 + 0.866 \log [Pb]_{total} - 0.689 pH_{CaCl_2} + 0.726 \log CEC - 2.16 \log WHC + 0.741 \log OM$	0.922	<0.001
$\log C_{body} = -2.05 + 0.614 \log [Pb]_{CaCl_2 \text{ extractable}} + 0.837 \log CEC - 0.408 \log Ca$	0.847	<0.001
$\log C_{body} = 1.58 + 0.730 \log [Pb]_{pw} + 0.215 \log Ca$	0.828	<0.001
$\log C_{body} = -1.73 + 0.443 \log [Pb]_{free} + 0.473 \log Ca + 0.396 pH_{pw} + 0.634 \log DOC$	0.834	<0.001

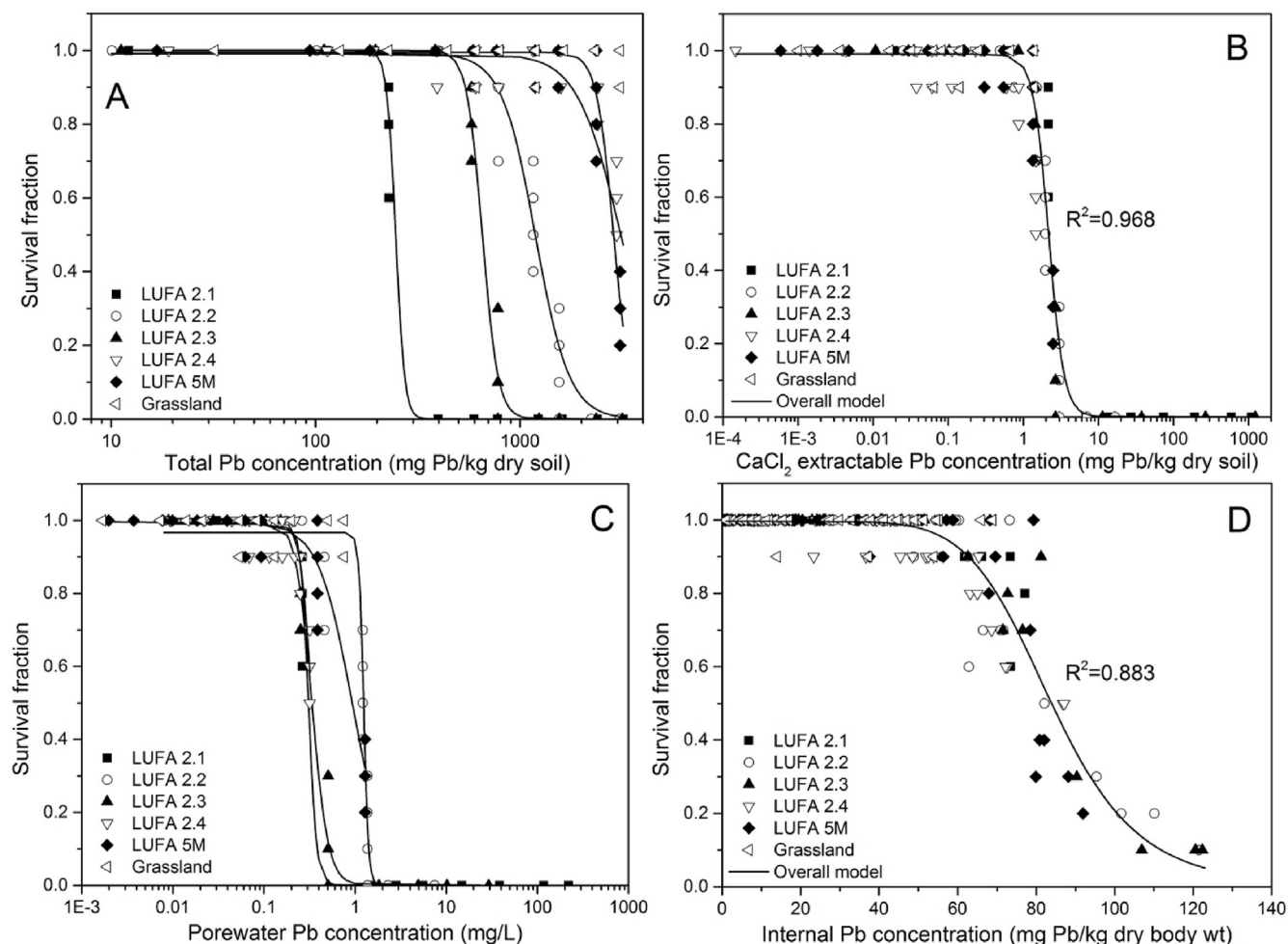


Fig. 2. Effects of $Pb(NO_3)_2$ on the survival of *Enchytraeus crypticus* after three weeks exposure in six soils with different soil properties. Pb concentrations are expressed as total (A) and 0.01 M $CaCl_2$ extractable concentrations in soil (B), concentrations in pore water (C) or internal concentrations in surviving adults (D). Lines show the fit of a logistic dose-response curve; in cases where only one curve is shown, the dose-response curves for soils did not significantly differ.

Grassland soil and LC10s from 221 mg Pb/kg dry soil in LUFA 2.1 to >3092 mg Pb/kg dry soil in Grassland soil (Table 2) and values differed significantly according to the generalized likelihood ratio test. Fitting a single dose-response curve to all survival data related to total soil concentrations gave a poor fit with $R^2 = 0.299$ (Fig. S4A).

In all soils, the number of juveniles produced decreased sharply with increasing total Pb concentration in the soil, which pattern was fitted well by the logistic dose-response model ($R^2 > 0.94$; Fig. 3). Estimated EC50 values based on total Pb concentration

varied 12-fold among soils and ranged from 81.4 mg Pb/kg dry soil in LUFA 2.1 to 1008 mg Pb/kg dry soil in LUFA 5 M. Also these values differed significantly according to the generalized likelihood ratio test. EC10s differed 8-fold from 51.6 mg Pb/kg dry soil in LUFA 2.1 to 438 mg Pb/kg dry soil in Grassland soil (Table 2). Pearson correlation analysis identified pH_{CaCl_2} and Ca concentration in the pore water as the most significant factors for Pb toxicity (Table 3). Fitting a single dose-response curve to all reproduction data related to total soil concentrations gave a poor fit with $R^2 = 0.598$ (Fig. S5A).

LC50, LC10, EC50 and EC10 values based on extractable Pb

Table 2
LC50, LC10, EC50 and EC10 values (with 95% confidence intervals) for the effects of lead on the survival and reproduction of *Enchytraeus crypticus* exposed for 21 days to Pb(NO₃)₂ in six different natural soils. Effect concentrations are expressed on the basis of total and 0.01 M CaCl₂-extractable Pb concentrations in soil, Pb concentrations in pore water and internal Pb concentrations measured in the surviving animals.

	LC50					LC10				
	Total Pb (mg/kg dry soil)	CaCl ₂ Extractable Pb (mg/kg dry soil)	Porewater Pb (mg/L)	Internal Pb (mg/kg body wt)	Total Pb (mg/kg dry soil)	CaCl ₂ Extractable Pb (mg/kg dry soil)	Porewater Pb (mg/L)	Internal Pb (mg/kg body wt)	Total Pb (mg/kg dry soil)	CaCl ₂ Extractable Pb (mg/kg dry soil)
Lufa 2.1	246 (–)	2.35 (–)	0.308 (–)	95.7 (60.8–131)	221 (–)	2.08 (–)	0.246 (–)	61.6 (45.7–77.6)	221 (–)	2.08 (–)
Lufa 2.2	1192 (1141–1243)	2.11 (2.02–2.20)	1.25 (1.23–1.27)	83.0 (79.3–86.7)	785 (704–869)	1.31 (1.19–1.42)	1.09 (1.05–1.16)	59.6 (54.6–64.6)	785 (704–869)	1.31 (1.19–1.42)
Lufa 2.3	655 (642–667)	1.86 (1.78–1.93)	0.335 (0.318–0.349)	87.0 (83.6–90.3)	540 (524–556)	1.24 (1.16–1.32)	0.213 (0.195–0.226)	66.5 (61.6–71.4)	540 (524–556)	1.24 (1.16–1.32)
Lufa 2.4	3125 ^a (3034–3216)	1.64 ^a (1.54–1.74)	0.334 ^a (0.325–0.343)	84.3 ^a (80.9–86.7)	1825 ^a (2158–2429)	0.797 ^a (0.700–0.895)	0.213 ^a (0.234–0.260)	59.4 ^a (52.8–59.5)	1825 ^a (2158–2429)	0.797 ^a (0.700–0.895)
Lufa 5 M	2875 (2820–2930)	2.11 (2.01–2.20)	0.933 (0.853–1.01)	81.7 (80.1–83.4)	2327 (2218–2435)	1.29 (1.15–1.43)	0.360 (0.284–0.435)	69.7 (65.7–73.7)	2327 (2218–2435)	1.29 (1.15–1.43)
Grassland	>3092 ^b	>1.39 ^b	>0.754 ^b	>47.7 ^b	>3092 ^b	>1.39 ^b	>0.754 ^b	>47.7 ^b	>3092 ^b	>1.39 ^b
	EC50					EC10				
	Total Pb (mg/kg dry soil)	CaCl ₂ Extractable Pb (mg/kg dry soil)	Porewater Pb (mg/L)	Internal Pb (mg/kg body wt)	Total Pb (mg/kg dry soil)	CaCl ₂ Extractable Pb (mg/kg dry soil)	Porewater Pb (mg/L)	Internal Pb (mg/kg body wt)	Total Pb (mg/kg dry soil)	CaCl ₂ Extractable Pb (mg/kg dry soil)
Lufa 2.1	81.4 (61.9–100)	0.329 (0.186–0.471)	0.044 (–)	13.6 (5.96–21.2)	51.6 (22.5–80.1)	0.143 (0.003–0.289)	0.021 (–)	5.08 (–)	51.6 (22.5–80.1)	0.143 (0.003–0.289)
Lufa 2.2	238 (224–251)	0.193 (0.160–0.211)	0.127 (0.120–0.133)	34.12 (30.6–37.7)	97.2 (85.5–108)	0.047 (0.031–0.056)	0.067 (0.053–0.069)	19.5 (15.3–23.6)	97.2 (85.5–108)	0.047 (0.031–0.056)
Lufa 2.3	205 (191–219)	0.107 (0.081–0.126)	0.117 (0.113–0.120)	26.0 (24.0–28.0)	102 (97.2–117)	0.017 (0.008–0.024)	0.090 (0.083–0.097)	15.0 (12.3–17.7)	102 (97.2–117)	0.017 (0.008–0.024)
Lufa 2.4	948 (801–1094)	0.180 (0.127–0.235)	0.169 (0.150–0.188)	39.9 (32.8–47.1)	198 (118–277)	0.009 (0.002–0.017)	0.065 (0.042–0.083)	12.5 (5.42–19.6)	198 (118–277)	0.009 (0.002–0.017)
Lufa 5 M	1008 (868–1148)	0.241 (0.166–0.309)	0.046 (0.034–0.059)	27.2 (22.1–32.5)	364 (243–484)	0.030 (0.007–0.051)	0.006 (0.002–0.011)	7.45 (3.93–11.0)	364 (243–484)	0.030 (0.007–0.051)
Grassland	991 (930–1052)	0.115 (0.098–0.125)	0.105 (0.094–0.116)	32.6 (28.3–36.9)	438 (376–499)	0.036 (0.024–0.043)	0.038 (0.025–0.043)	16.6 (10.5–22.8)	438 (376–499)	0.036 (0.024–0.043)

- Data did not allow calculating reliable LC50, LC10 and 95% confidence intervals.

^a Less than 50% mortality at the highest exposure concentration, but data still allowed calculation of LC50s.

^b Less than 10% mortality at the highest exposure concentration, so data did not allow calculating LC50s.

concentration (CaCl₂-extractable, porewater) and internal Pb concentrations are presented in Table 2. The difference in LC50s and EC50s between the different soils could be well explained by CaCl₂-extractable Pb concentrations, allowing to fit a single overall dose-response curve to the joint data for all test soils ($R^2 > 0.943$) (Figs. 2B and 3B). LC50 values based on Pb concentrations in the pore water ranged from 0.308 to 1.25 mg/L. When mortality and reproduction data for all soils related to porewater concentrations were fitted with a single dose-response curve, R^2 was 0.878 and 0.863, respectively (Figs. S4B and S5B). Internal Pb concentration could well describe enchytraeid mortality data from all test soils, allowing the fit of a single overall dose response curve ($R^2 = 0.883$) (Fig. 2D). Fitting reproduction data as a function of internal Pb concentrations with a single dose-response curve gave an R^2 of 0.837 (Fig. S5C), but better fits were obtained when using separate curves for each soil type (Fig. 3D).

4. Discussion

4.1. Soil properties and Pb extractability

The addition of Pb caused a dose-related pH decrease in soils spiked with Pb(NO₃)₂, which was also observed previously (Zhang and Van Gestel, 2017a). Soils with low OM content and CEC (LUFA 2.1 and LUFA 2.3) showed a stronger decrease in soil pH at higher Pb concentrations than the high OM content and high CEC soils (LUFA 2.2, LUFA 2.4, LUFA 5 M and Grassland soil), which apparently had a higher buffer capacity.

Extractable Pb concentrations (CaCl₂-extractable and porewater concentrations, porewater Pb²⁺ free ion activity) are dependent not only on total Pb concentration but also on soil characteristics, such as the sorption phase (clay, OM, hydroxides), the number of binding sites (CEC) and the pH of the soil (Janssen et al., 1997). The partition coefficient K_F well described Pb sorption to our test soils. K_{FC} values for Pb sorption related to CaCl₂-extractable Pb concentrations increased with increasing OM content, CEC and WHC. OM content best predicted K_{FC} values (Table S9), which agrees with soil organic matter acting as a crucial sink for Pb (Sánchez-Camazano et al., 1998). Romero-Freire et al. (2015) reported that soils with higher organic carbon and pH had lower extractable Pb concentrations, confirming the important roles of pH and organic carbon in controlling Pb partitioning in soils. Besides CEC, pH_{pw} also was an important factor for K_{FP} based on porewater Pb concentration. This agrees with the finding that soil pH affected Pb availability because it determines the solubility and speciation of Pb in the soil (Smolders et al., 2009). In general, the regression analysis highlighted the importance of CEC explaining over 80% of the variation in Pb sorption based on the extractable Pb concentrations in the six test soils. The CEC of soils is determined by clay type and clay content, and the presence of organic matter and/or hydrous oxides to which Pb has a strong affinity (Phillips, 1999).

4.2. Pb uptake in enchytraeids

After 21 days of exposure, the Pb concentrations in the enchytraeids linearly increased with increasing external Pb concentration for all six test soils ($R^2 > 0.69$) but showed large differences between soils and did not seem to level off to a maximum in some soils. However, when relating internal Pb concentrations to extractable Pb concentrations (CaCl₂-extractable or porewater Pb concentration), a maximum Pb uptake was found, estimated to be 82.9 and 95.7 mg Pb/kg dry body wt, respectively using overall Langmuir models. This is consistent with the observation of Zhang and Van Gestel (2017a), who reported that the maximum uptake

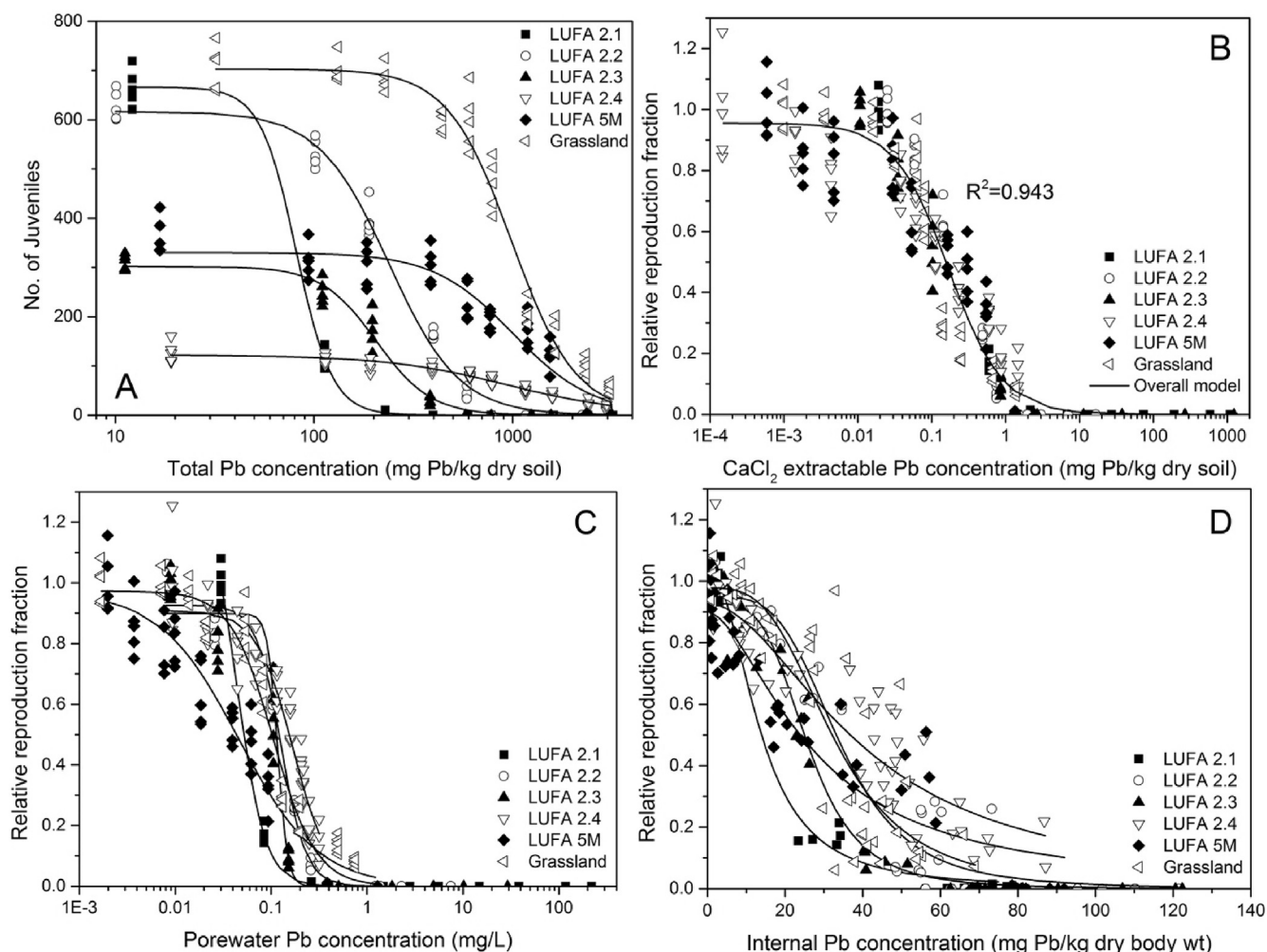


Fig. 3. Effects of $\text{Pb}(\text{NO}_3)_2$ on the reproduction of *Enchytraeus crypticus* after three weeks exposure in six natural soils. Pb concentrations are expressed as total (A) and 0.01 M CaCl_2 extractable concentrations in soil (B), concentrations in pore water (C) or internal concentrations in the surviving adults (D). Lines show the fit of a logistic dose-response curve; in cases where only one curve is shown, the dose-response curves did not significantly differ.

Table 3

Simple and multiple stepwise regressions of LC50, LC10, EC50 and EC10 values for Pb toxicity to *Enchytraeus crypticus* based on total Pb concentration in six different natural soils with different soil properties. R^2 (adj) presents the percentage of variation explained by only the independent variables that actually affect the dependent variable. See Table 2 for Pb toxicity data and Table S1 for soil properties.

Regression type	Dependent variable	N =	R^2 (adj)	Equation	p
Simple	LC50	5	0.934	$\log \text{LC50} = -0.084 + 1.23 \log \text{Ca}$	0.005
Simple	LC50	5	0.941	$\log \text{LC50} = 1.99 + 1.30 \log \text{CEC}$	0.004
Simple/stepwise	LC50	5	0.975	$\log \text{LC50} = 0.024 + 0.519 \text{pH}_{\text{CaCl}_2}$	0.001
Simple	LC10	5	0.785	$\log \text{LC10} = 2.05 + 1.05 \log \text{CEC}$	0.029
Simple	LC10	5	0.911	$\log \text{LC10} = 0.438 + 0.427 \text{pH}_{\text{CaCl}_2}$	0.008
Simple/stepwise	LC10	5	0.990	$\log \text{LC10} = 0.058 + 1.13 \log \text{Ca}$	<0.001
Simple	EC50	6	0.843	$\log \text{EC50} = 1.56 + 1.14 \log \text{CEC}$	0.006
Simple	EC50	6	0.872	$\log \text{EC50} = 0.245 + 1.01 \log \text{Ca}$	0.004
Simple/stepwise	EC50	6	0.969	$\log \text{EC50} = -0.334 + 0.484 \text{pH}_{\text{CaCl}_2}$	<0.001
Simple	EC10	6	0.646	$\log \text{EC10} = 1.28 + 0.802 \log \text{CEC}$	0.033
Simple	EC10	6	0.817	$\log \text{EC10} = 0.224 + 0.888 \log \text{Ca}$	0.008
Simple/stepwise	EC10	6	0.839	$\log \text{EC10} = 0.009 + 0.361 \text{pH}_{\text{CaCl}_2}$	0.007

capacity of *E. crypticus* ranged from 76.6 to 90.5 mg Pb/kg dry body based on Pb extractability in soils. Davies et al. (2003) found that earthworms were able to regulate internal Pb concentration at low soil Pb concentrations but uptake linearly increased at Pb concentrations higher than 3000 mg Pb/kg dry soil. The limitation of Pb accumulation at high exposure concentrations in our study could

partly be explained by the biotic ligand model (BLM). In our study, porewater Ca concentration and H^+ activity increased with increasing soil Pb concentrations. The BLM assumes that cations (e.g. H^+ and Ca^{2+}) in the soil solution compete with metal ions (e.g. Pb^{2+}) for the binding sites on the organisms, leading to a protection from metal uptake at high exposure concentrations (Ardestani

et al., 2015). This also explained the fact that accounting for the presence of protons (H^+) improved the prediction of internal Pb concentrations from free Pb^{2+} activity in pore water (Fig. 1D). Furthermore, the good correlation between Pb accumulation in *E. crypticus* and Pb extractability ($CaCl_2$ extractable and porewater Pb concentration) suggested that the differences between soils might be minimized when relating Pb bioaccumulation to extractable instead of total soil Pb concentrations (Fig. 1). This also revealed that the bioavailable pool in soil for Pb uptake in enchytraeids was the $CaCl_2$ -extractable or porewater fraction. This is in line with the notion that pore water is the major route for metal uptake in soil invertebrates (Van Gestel, 2012). Therefore, free Pb^{2+} ion activity in the soil solution, as a function of total metal content, was regarded to be the extractable fraction for uptake by soil invertebrates. In present study, free Pb^{2+} ion activity, however, could explain only 46% of the variance in Pb accumulation, which was much lower than the other extractable Pb fractions (79.5% and 81.9% for $CaCl_2$ extractable and porewater Pb concentrations, respectively), suggesting that other Pb forms, existing in the pore water (uptake) or on the surface of soil particles (ingestion), might also contribute to Pb uptake by soil invertebrates (Luo et al., 2014a).

Internal Pb concentrations in *E. crypticus* were well predicted by total Pb concentrations, pH, CEC, WHC and OM content of the soils, describing 92.2% of the variance. These factors affected Pb extractability in our test soils and subsequently influenced Pb accumulation in worms. Thus, soil properties (e.g. OM content, pH_{CaCl_2} and CEC) might have direct or indirect effects on body Pb concentrations in enchytraeids. This partly agrees with previous studies, suggesting that soil pH was the most important factor controlling Pb bioavailability in soil followed by OM content (Luo et al., 2014b; Peijnenburg et al., 1999b).

4.3. Pb toxicity in relation to soil properties

In the present study, CEC, pH_{CaCl_2} and Ca concentrations in pore water were the factors most significant for Pb toxicity (LC50 and LC10) according to Pearson correlation analysis. Simple and stepwise regression analysis showed that pH_{CaCl_2} was best explaining LC50 values based on total Pb concentration, while Ca concentration in the pore water was the dominant factor affecting LC10 values. This agrees with previous studies where pH was the most important soil property influencing Pb toxicity to the survival of soil organisms (Bradham et al., 2006; Luo et al., 2014a; Wijayawardena et al., 2017). Although Grassland soil and LUFA 2.4 had similar high pH_{CaCl_2} and CEC values, toxicity was lower in Grassland soil (<5% mortality at the highest exposure level). This difference might result from the fact that, compared to LUFA 2.4, Grassland soil had higher porewater Ca concentrations. Ca^{2+} might compete with Pb^{2+} for binding sites on the organisms, resulting in a decrease of Pb bioavailability. Mortality of the organisms could only be observed when body Pb concentration exceeded a physiological limit (lethal body concentration) (Jager et al., 2011; Vijver et al., 2004). Zhang and Van Gestel (2017b) reported a lethal body concentration of 66 mg Pb/kg dry body wt for *E. crypticus*, which is much higher than the body concentrations determined in the Grassland soil. So, besides pH_{CaCl_2} , porewater Ca concentration also played an important role in explaining Pb toxicity in the test soils.

Although no enchytraeid mortality was observed in the control soils, the juvenile numbers greatly differed among the soils tested. Compared the other soils, reproduction in LUFA 2.4 was lowest, which may be attributed to the very high clay content and relatively low OM content. Van Gestel et al. (2011) found that a soil with high clay and low organic carbon content was unsuitable for earthworms to reproduce. Thus, soil properties, such as clay content and OM content, might have influenced the reproduction of the

enchytraeids. CEC, pH_{CaCl_2} and porewater Ca concentration were identified as the factors determining EC50 and EC10 based on total Pb concentration. This agrees with the findings of Luo et al. (2014b), who found that Ca, pH and CEC were the most important soil properties modifying enchytraeid reproduction in Pb contaminated field soils. With different soil characteristics (e.g. CEC, OM content and clay content) but similar pH_{CaCl_2} , LUFA 2.4, LUFA 5 M and Grassland soil had similar EC50 values, indicating that pH_{CaCl_2} was the main factor describing Pb toxicity to enchytraeid reproduction. This was confirmed by the simple and stepwise regression analysis, and by other authors (Luo et al., 2014a; Wijayawardena et al., 2017).

Despite the greatly different LC50 values based on total Pb concentrations among soils with different properties, the LC50s expressed on the basis of $CaCl_2$ -extractable Pb concentrations in soil or internal Pb concentrations in the enchytraeids did not differ much. Survival fraction could well be described by $CaCl_2$ -extractable and internal Pb concentrations for all test soils ($R^2 > 0.88$) and were independent of soil type (Fig. 2B and D). Some other authors also confirmed that $CaCl_2$ -extractable Pb concentrations better predict Pb toxicity for enchytraeids than total soil Pb concentrations (Lock and Janssen, 2001; Luo et al., 2014b). In a previous study, we also observed that survival fraction well correlated with body Pb concentrations in *E. crypticus* when exposed to different Pb salts (Zhang and Van Gestel, 2017a). Eliminating effects of different routes of uptake, internal Pb concentrations therefore might be a good indicator of Pb bioavailability and toxicity in soils.

The reproduction of *E. crypticus* was best described by $CaCl_2$ -extractable Pb concentrations for all test soils (Fig. 3B), which is consistent with the finding that extractable Pb concentration was a better measure of Pb toxicity to reproduction than total, porewater or internal Pb concentrations when exposed to different Pb salts amended soils (Zhang and Van Gestel, 2017a). Therefore, the variation in EC50 was best explained by differences in $CaCl_2$ -extractable Pb concentration in soils.

5. Conclusion

Differences in Pb extractability, uptake and toxicity to *E. crypticus* were observed in Pb spiked soils with different soil properties. Pb sorption to the soils was mainly affected by CEC. Pb uptake in *E. crypticus* was positively correlated with exposure Pb concentrations and best explained by Pb extractability in the soils measured as $CaCl_2$ -extractable and porewater Pb concentrations. CEC, Ca concentration in pore water and pH also affected Pb bioaccumulation in the enchytraeids. Toxicity (LC50, LC10, EC50 and EC10) based on total Pb concentrations varied considerably among soils. Although CEC and Ca concentration in pore water influenced Pb toxicity to *E. crypticus*, pH_{CaCl_2} was the dominant factor predicting LC50, LC10, EC50 and EC10 values in simple and stepwise regression analysis. Overall, survival and reproduction effects were best explained from $CaCl_2$ -extractable Pb concentrations. This study shows that soil properties are important factors modifying metal bioavailability to soil organisms and should be considered during the ecological risk assessment of metals in contaminated soils.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2018.10.146>.

References

- Antunes, S.C., Castro, B.B., Pereira, R., Gonçalves, F., 2008. Contribution for tier 1 of the ecological risk assessment of Cunha Baixa uranium mine (Central Portugal): II. Soil ecotoxicological screening. *Sci. Total Environ.* 390, 387–395.
- Ardestani, M.M., Van Straalen, N.M., Van Gestel, C.A.M., 2015. Biotic ligand modeling approach: synthesis of the effect of major cations on the toxicity of metals to soil and aquatic organisms. *Environ. Toxicol. Chem.* 34, 2194–2204.
- Bradham, K.D., Dayton, E.A., Basta, N.T., Schroder, J., Payton, M., Lanno, R.P., 2006. Effect of soil properties on lead bioavailability and toxicity to earthworms. *Environ. Toxicol. Chem.* 25, 769–775.
- Castro-Ferreira, M.P., Roelofs, D., Van Gestel, C.A.M., Verweij, R.A., Soares, A.M.V.M., Amorim, M.J.B., 2012. *Enchytraeus crypticus* as model species in soil ecotoxicology. *Chemosphere* 87, 1222–1227.
- Conder, J.M., Lanno, R.P., 2000. Evaluation of surrogate measures of cadmium, lead, and zinc bioavailability to *Eisenia fetida*. *Chemosphere* 41, 1659–1668.
- Davies, N.A., Hodson, M.E., Black, S., 2003. The influence of time on lead toxicity and bioaccumulation determined by the OECD earthworm toxicity test. *Environ. Pollut.* 121, 55–61.
- Dohrmann, R., 2006. Cation exchange capacity methodology II: a modified silver–thiourea method. *Appl. Clay Sci.* 34, 38–46.
- Facchinelli, A., Sacchi, E., Mallen, L., 2001. Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. *Environ. Pollut.* 114, 313–324.
- Gustafsson, J.P., 2011. Visual MINTEQ 3.0 User Guide. KTH, Department of Land and Water Resources, Stockholm, Sweden.
- ISO, 2012. Determination of the Inhibition of the Mobility of *Daphnia magna* Straus (*Cladocera, Crustacea*) – Acute Toxicity Test. ISO 6341. International Organization for Standardization, Geneva, Switzerland.
- Jager, T., Albert, C., Preuss, T.G., Ashauer, R., 2011. General unified threshold model of survival-a toxicokinetic-toxicodynamic framework for ecotoxicology. *Environ. Sci. Technol.* 45, 2529–2540.
- Janssen, R., Peijnenburg, W.J.G.M., Posthuma, L., Van Den Hoop, M.A.G.T., 1997. Equilibrium partitioning of heavy metals in Dutch field soils. I. Relationship between metal partition coefficients and soil characteristics. *Environ. Toxicol. Chem.* 16, 2470–2478.
- Lock, K., Janssen, C.R., 2001. Test designs to assess the influence of soil characteristics on the toxicity of copper and lead to the oligochaete *Enchytraeus albidus*. *Ecotoxicology* 10, 137–144.
- Luo, W., Verweij, R.A., Van Gestel, C.A.M., 2014a. Assessment of the bioavailability and toxicity of lead polluted soils using a combination of chemical approaches and bioassays with the collembolan *Folsomia candida*. *J. Hazard. Mater.* 280, 524–530.
- Luo, W., Verweij, R.A., Van Gestel, C.A.M., 2014b. Contribution of soil properties of shooting fields to lead bioavailability and toxicity to *Enchytraeus crypticus*. *Soil Biol. Biochem.* 76, 235–241.
- OECD, 2016. Guidelines for the Testing of Chemicals No. 220–Enchytraeid Reproduction Test. Organisation for Economic Co-operation and Development, Paris, France.
- Peijnenburg, W.J.G.M., Baerselman, R., de Groot, A.C., Jager, T., Posthuma, L., Van Veen, R.P.M., 1999a. Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the oligochaete *Eisenia andrei*. *Ecotoxicol. Environ. Saf.* 44, 294–310.
- Peijnenburg, W.J.G.M., Posthuma, L., Zweers, P.G.P.C., Baerselman, R., de Groot, A.C., Van Veen, R.P.M., Jager, T., 1999b. Prediction of metal bioavailability in Dutch field soils for the oligochaete *Enchytraeus crypticus*. *Ecotoxicol. Environ. Saf.* 43, 170–186.
- Peijnenburg, W.J.G.M., Zablotzka, M., Vijver, M.G., 2007. Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. *Ecotoxicol. Environ. Saf.* 67, 163–179.
- Phillips, I.R., 1999. Copper, lead, cadmium, and zinc sorption by waterlogged and air-dry soil. *J. Soil Contam.* 8, 343–364.
- Romero-Freire, A., Peinado, F.J.M., Van Gestel, C.A.M., 2015. Effect of soil properties on the toxicity of Pb: assessment of the appropriateness of guideline values. *J. Hazard. Mater.* 289, 46–53.
- Sánchez-Camazano, M., Sanchez-Martin, M.J., Lorenzo, L.F., 1998. Significance of soil properties for content and distribution of cadmium and lead in natural calcareous soils. *Sci. Total Environ.* 218, 217–226.
- Smith, B.A., Greenberg, B., Stephenson, G.L., 2012. Bioavailability of copper and zinc in mining soils. *Arch. Environ. Contam. Toxicol.* 62, 1–12.
- Smolders, E., Oorts, K., Van Sprang, P., Schoeters, I., Janssen, C.R., McGrath, S.P., McLaughlin, M.J., 2009. Toxicity of trace metals in soil as affected by soil type and aging after contamination: using calibrated bioavailability models to set ecological soil standards. *Environ. Toxicol. Chem.* 28, 1633–1642.
- Van Gestel, C.A.M., 2012. Soil ecotoxicology: state of the art and future directions. *ZooKeys* 275.
- Van Gestel, C.A.M., Borgman, E., Verweij, R.A., Ortiz, M.D., 2011. The influence of soil properties on the toxicity of molybdenum to three species of soil invertebrates. *Ecotoxicol. Environ. Saf.* 74, 1–9.
- Vijver, M.G., Van Gestel, C.A.M., Lanno, R.P., Van Straalen, N.M., Peijnenburg, W.J.G.M., 2004. Internal metal sequestration and its ecotoxicological relevance: a review. *Environ. Sci. Technol.* 38, 4705–4712.
- Wijayawardena, M.A.A., Megharaj, M., Naidu, R., 2017. Bioaccumulation and toxicity of lead, influenced by edaphic factors: using earthworms to study the effect of Pb on ecological health. *J. Soils Sediments* 17, 1064–1072.
- Zhang, L., Van Gestel, C.A.M., 2017a. The toxicity of different lead salts to *Enchytraeus crypticus* in relation to bioavailability in soil. *Environ. Toxicol. Chem.* 36, 2083–2091.
- Zhang, L., Van Gestel, C.A.M., 2017b. Toxicokinetics and toxicodynamics of lead in the soil invertebrate *Enchytraeus crypticus*. *Environ. Pollut.* 225, 534–541.